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SCIENCE

In This Issue

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Dean, American University, Washington, D.C.

THE UNIVERSITY STUDY CENTER and the Student Detachment, University of Florence, Italy, known jointly and officially as the University Training Command, Mediterranean Theater of Operations (UTC, MTOUSA), represent the earliest experiment in establishing Army universities overseas. The University Study Center in Florence originated in 1943, when the War Department sought to provide educational opportunities for American military personnel in the Mediterranean Theater. As in the European Theater, concern was evidenced for the large number of troops that would necessarily remain in Italy and Africa for some time after V-E Day while awaiting their redeployment or discharge. Accordingly, plans were initiated from Washington in November 1944 for a university in the Mediterranean Theater. The UTC was activated under Brig. Gen. Foster J. Tate, former professor of military science and tactics at the Virginia Military Institute, and the first session of the University began on 1 July 1945.

Administration

Unlike the sister Army universities in the European Theater of Operations, Shrivenham (England) and Biarritz (France), the UTC at Florence was organized and operated in its opening session exclusively by military personnel recruited from within the Mediterranean Theater. Requests had been made for the names of military personnel with prewar backgrounds of educational activities, and the original staff was selected on the basis of academic qualifications. At the close of the first session the Commandant, Brig. Gen. Tate, was transferred to Washington and was succeeded by Col. John W. Harmony (Inf.), formerly of the U. S. Military Academy. The Commandant acted as president of the University Study Center, and an Assistant Commandant, Col. Irving C. Whittemore (CAC), formerly professor at the Boston University School of Business Administration, functioned as vice-president. Col. Wentworth Williams (Inf.), associate professor at Boston University School of Business Administration and a graduate of the Command and General Staff School, performed the role of dean. Capt. Verna A. McCluskey (WAC, AC) was dean of women, caring for the limited enrollment of WAC's and Army Nurses. Capt. Gordon C. Atkins (AC) was secretary of the faculty. The administration was rounded by the additions of Adjutant General, who was registrar; assistants to the president; assistant deans (for general counseling services); officers in charge of athletics and recreation; librarian; and Commandants of Troops, Military Operations and Services, Supply, and Finance, with their numerous assistants and office staffs, all of whom were military personnel.

Campus

The campus was the spacious Fascist School of Applied Aeronautics, built in 1938 and consisting of many large modern buildings well suited for the demands of instruction. The campus was beautifully landscaped and situated in the equally beautiful Casine Park, about two miles from the center of Florence. The buildings were named to preserve the traditions of home and were officially called Harvard, Yale, Princeton, Cornell, Duke, Vanderbilt, and Stanford, with innumerable temporary annexes. There were 25 modern classrooms in the official buildings, administrative offices, and Red Cross Center. Within the campus were athletic fields, gymnasium, swimming pool, riding track, etc. The station complement numbered 500, and in addition some 400 POW's worked as janitors and kitchen help. Students not billeted at the University Study Center were quartered in Florence, in the former 5th Army Rest Center, which was the recently completed, thoroughly modernized railroad station, now inoperative. The faculty and administrative staff were housed in the city, in hotels which acted as residential clubs. The physical organization of the university, the provision for instruction and residence, was uniquely modern and up to date for efficient and effective university life.

Personnel

The Administration consisted of 11 officers, their assistants, and complement. Of this number three held the Ph.D. degree; two were graduates of the U. S. Military Academy with the B.S. degree; three others held M.A., M.S., and M.Litt. degrees, respectively; one had a B.S.; one, a B.A.; and one held no degree.

The teaching faculty consisted of 219 members ranging from privates to lieutenant colonels. Of this number, 28 were doctors; 65, masters (24 with additional graduate study); and 83, bachelors (15 with additional graduate study).

With the beginning of the second session on 5 August, civilian instructors were called from colleges, universities, and government bureaus in the United States to augment the rapidly expanding program. By the end of this session and the beginning of the third, 41 additional instructors were conducting classes. The home colleges and universities from which they came included: North Carolina, Connecticut State, Iowa, Goucher, Los Angeles City College, American, Duke, West Virginia, Chicago, George Washington, South West Missouri, Miami, Minnesota, Georgia, Massachusetts State, City College of New York, Nebraska, Yale, Manchester, Pomona, Missouri, North Carolina State, Knox, Hunter, Wilson State Teachers, Wayne, Kentucky, and Louisiana State.

A distribution of their academic and professional status shows that of this total there were 30 Ph.D.'s, 7 M.A.'s, 1 M.B.A., 1 M.S., 1 B.S. (Agricultural), and 1 unclassified (Art), and that the number comprised 17 professors, 7 associate professors, 5 assistant professors, 1 teaching fellow, 2 lecturers, 1 business manager, 7 government employees, and 1 who was unclassified.

The entire faculty, including the Administration, consisted of 31 doctors, 68 masters, and 86 bachelors.

Student Selection

The admission of students to the University Study Center was made on a reasonably high selective rating. The minimum admission requirements specified high school graduation, some previous college training, or a minimum Army General Classification Test score of 105 points. This latter requirement was five points less than the number required for attendance at an Officer Candidate School. Students were selected by their own Army units and sent on the basis of quotas allotted to major commands by the UTC.

Sessions and Curricula

An official session for attendance at the Study Center was four weeks. Each fifth week was used to complete all military and academic records, return to their units men who were not remaining for the ensuing session, and receive and billet new students. All courses met for a period of 55 minutes five days each week, Saturday morning being left for one-hour orientation lectures on current political and related events. In addition, there was one daily period of physical training. A student taking two sessions earned approximately the equivalent of a three-semester-hour credit for each course taken. The normal teaching load for the faculty was three courses per session.

The academic calendar consisted of four sessions: 2 July–5 August, 8 August–9 September, 12 September–14 October, and 17 October–17 November 1945. The Student Detachment at the University of Florence began on 9 August and ended on 27 October 1945.

Courses were offered under the Departments of Agriculture, Biological Sciences, Business Administration, English, Education, Fine Arts, Languages, Mathematics, Physical Sciences, Social Sciences (including economics, history, philosophy, political science, psychology, and sociology), and Physical Education. Those offered the Student Detachment at the University of Florence included Fine Arts, Languages, Literature, Science, Social and Political Science (including civil and commercial law, criminal and constitutional law), and Psychology.

Educational Opportunities in the Sciences

Biological sciences. The Department of Biological Sciences at the University Study Center was staffed by 13 instructors—4 officers, 6 sergeants, and 3 civilian instructors, as follows:

Maj. Frank H. Connell, SnC (chairman, Session I), Zoology; Capt. Milton F. Kernkamp, CMP (chairman, Sessions II, III), Biology; Henry W. Olson (chairman, Session IV), Biology; Maj. Robert J. Reedy, SnC, Bacteriology; 1st Lt. James A. Green, QMC; Sgts. Hugh C. Sauer, William C. Beckman, Beryl F. Capps, John L. Stewart, Martin J. Ulmer, and Alan D. Randall, WOJG; Augustus T. Miller, Jr., and Archibald W. Bell.

Lectures and some laboratory work were offered in the University Study Center, but the greater part of the laboratory courses were given in the University of Florence. The Rector Magnificus, Prof. Piero Calamandrei, had generously offered the use of whatever libraries, classrooms, and laboratories were needed, and through his official cooperation and vigorous assistance the Study Center was able to offer some 40 courses in geology, physics, chemistry, zoology, botany, biology, bacteriology, and psychology. The University of Florence was relatively well supplied with microscopes, slides, models, charts, etc. In the University Museum was a collection of wax models which is probably the finest exhibit of its kind in the world. For

the study of anatomy this collection is superb. Every organ, muscle, and system is portrayed in different aspects and in various degrees of dissection. The Museum was open constantly for observation.

The bacteriology classes were held in a very modern laboratory building provided with excellent equipment. The Museum of Zoology offered a fine collection of vertebrate and invertebrate animals gathered from all over the world. The botany library likewise was considered one of the finest in Europe, and the Herbarium, one of the largest. Courses in General Biology (I, II), Botany (I, II), Zoology (I, II), Physiology (I, II), Anatomy (I, II), and Bacteriology (I, II), were offered biological students by the Study Center.

Physical sciences. The Department of Physical Sciences, consisting of physics, chemistry, geology, and mineralogy, was administered by a staff of 21 instructors—13 officers, 5 sergeants, and 3 civilian instructors, as follows:

Capt. Ulysses S. Jones, Jr., FA (chairman, Sessions I, II, III), Chemistry; Capt. Ludwig Audrieth, Ord. (chairman, Session IV), Chemistry; Maj. Kenneth W. Glace, QMC, Chemistry; Maj. Claude V. Pevey, CE, Physics; Capt. Harold E. Calbert, Inf., Chemistry; Capt. Nelson A. Terhune, SC, Physics; Capt. Thomas S. Schreiber, SC, Physics; Capt. Isadore Zipkin, SnC, Chemistry; 1st Lt. Robert M. Crisler, Inf., Geology; 1st Lt. George E. Prichard, Inf., Geology; 1st Lt. William S. Morton, Ord., Chemistry; 1st Lt. William J. Jackson, Jr., SC, Physics; 1st Lt. Jerome Saldick, AC, Chemistry; T/5 Sgts. George V. Hill, Physics; Joseph P. Larocca and Robert B. Power, Chemistry; and Claude Quigley, Geology; S/Sgt. Henry E. Wendon, Geology; Walter D. Keller, Geology; William W. Mutch, Physics; and Victor A. Goedicke, Astronomy.

The physics laboratories at the University of Florence were used by students enrolled in the University Study Center. These laboratories were well equipped with apparatus for the usual experimental work in general physics, including mechanics, sound, heat, electricity, and optics. The famous Florentine Observatory, where Galileo did most of his work, was also at the disposal of the students together with a library of rare books, including Galileo's original notes and telescope.

Three chemistry laboratories at the University of Florence (qualitative, general, and organic), with all reagents necessary, were available to the students at the Study Center. The library contained volumes rarely available in the United States.

The entire physical plant of the Instituto di Geologia e Paleontologia of the University was placed at the disposal of the UTC. The plant consisted of a large, three-story building housing the Museum of Vertebrate Paleontology, several thousand mounted and articulated vertebrate specimens, and life groups, with individual exhibits in the Museum of Invertebrate Paleontology. Over 100,000 fossils, ranked according to geologic age and obtained from the sedimentary deposits of all continents, were uniquely arranged. The Regional Petrography Collections, in which are included suites of specimens from the major localities in Europe and Asia, such as the famous Simplon Tunnel section, completed the geologic stock in trade. Teaching aids included a collection of rock and fossil specimens for classroom use, a balopticon and projection machine, a large collection of geologic and paleontologic wall charts, a set of U. S. Geological Survey folios and topographic maps, topographic maps of Italy, block models, and photographs. A library of 12,000 volumes in this building was ably supplemented by scientific periodicals from the United States, Great Britain, Germany, Italy, France, and Japan. Unfortunately, many of these did not pass beyond the year 1939. This was typical of most of the scientific books and journals, due to prohibitions placed on current literature by the erstwhile fascist administrations.

For the large class enrollments a spacious hall seating 75 students was used; for smaller classes, rooms with seating capacities of 25 students.

Another factor contributing to the success of the geologic studies was the cooperation given by the director of the Astronomical Institute in making available the University Observatory, to which regular trips were made. Field trips took the students to the valley of the Mugnone River, north of Florence, and enabled students to acquaint themselves with some aspects of the regional sedimentary geology and structure and to study the physiography of the region, with its excellent examples of various stages of the fluvial cycle. Advanced students—those who were permitted to extend their residence beyond one session—were honored with trips to Perugia, with its fossils and erosional features. Numerous famous localities were within motoring distance, such as the marble quarries of Carrar, the volcanic regions of the Alban Hills, Naples with its Vesuvius, and the mercury deposits of Monte Amiata.

For studies in mineralogy the facilities of the Instituto di Mineralogia, Petrologia e Geochimica were open to the UTC, under the same conditions that prevailed in the case of the Instituto di Geologia e Paleontologia. The Institute is housed in a large building adjoining that of the Institute of Geology and contains a well-mounted and labeled collection of petrographic types, a library of 8,000 volumes, important mineralogic periodicals up to the year 1939, and laboratories. A spectrograph, petrographic microscopes, numerous wall charts, and crystal models in glass were on hand.

The courses offered in the Department of Physical Sciences were: Introduction to College Chemistry (I, II, III), Organic Chemistry (I, II, III), Qualitative Chemical Analysis, Physical Chemistry, A Beginning Course in Physical Geology, Mineralogy, Physical Geography, Historical Geology, General Physics (I, II, III, IV), Survey of Physical Science (I, II), Basic Electricity, and Astronomy (I).

Psychology. The enrollment in psychology at the Study Center was particularly heavy. The courses consisted of Introduction to Psychology (I, II), Child Psychology (I, II), Psychology of Adjustment (I, II), Abnormal Psychology (I, II), and Psychology of Marriage and the Family. This last-named course and the course in Child Psychology were given by popular demand and apparent need on the part of the students, many of whom had left at home young families and others of whom were contemplating marriage on their return. The course on marriage was not offered until the third session. Three hundred students enrolled, and when the course was repeated in the fourth and last session, 775 joined its ranks.

The psychology courses were listed under the Department of Social Sciences, together with economics, history, philosophy, political science, and sociology, under the direction of Lt. Col. John H. Hougen, JAGD. In the Student Detachment at the University of Florence, psychology was included in the semester's curriculum. The Institute of Psychology, with its spacious laboratory located at Via Cesare Battisti, provided space for lectures and laboratory facilities. These courses were conducted mainly by the staff in psychology at the University of Florence. At the University Study Center there was no provision for experimental psychological studies. The staff consisted of 5 officers and 2 civilian instructors, as follows:

Lt. Col. John H. Hougen, JAGD (chairman, Sessions I, II, III, IV), Law; Lt. Col. Louis L. McQuitty, AGD, Applied Psychology; Capt. Kenneth S. Hitch, AGD, General Psychology; Maj. Raymond Sobel, MC, Abnormal Psychology; 1st Lt. Jacob S. Kounin, AGD, General Psychology; Lester A. Kirkendall, Psychology of Marriage; and John E. Bentley, General and Child Psychology.

Course 1000. A limited number of students with advanced qualifications were allowed to register for what was known as Course 1000, offered by all departments at the University Study Center. This course permitted individual study under the guidance of a faculty member, approved in advance by the department chairman and the dean.

Enrollment and Quality of Students

The Study Center, working on a minimum-month-session, in the course of its entire history of four sessions, or four months, enrolled 8,150 students. Each session averaged approximately 2,000 students. The Student Detachment at the University of Florence enrolled 350 students. The aggregate for students in the biological and physical sciences at the Study Center was as follows:

		Biological Sciences	*Physical Sciences*
Session	I	55	247
	II	167	399
	III	160	309
	IV	85	164
Totals		467	1,119

The registration in psychology for classes with an enrollment over 100 was:

		General Psychology	*Psychology of Marriage and the Family*
Session	I	186	Not offered
	II	310	Not offered
	III	295	300
	IV	236	775

The total enrollment in all classes in psychology for the four sessions, including those with less and those with more than 100 students, was 2,330.

With few exceptions the students were capable and deeply interested. Their war experiences had matured them both physically and mentally, and the majority were anxious to learn and prepared to work hard. As the dean (Col. Williams) said from time to time: "The men know what they want and demand it." The results of their efforts were noticeably commendable.

Association Affairs

Hotel Headquarters, Boston

General Headquarters: The Statler Hotel will serve as the general headquarters of the Association, housing the meetings of the Council and Executive Committee.

Headquarters of the sections of the Association and of the societies meeting with the Association follow:

Statler Hotel: Section on Medical Sciences (N), Subsections on Dentistry (Nd) and Pharmacy (Np); Academy Conference, American Microscopical Society, American Society of Naturalists, American Society of Parasitologists, American Society of Zoologists, Genetics Society of America, Ecological Society of America, Limnological Society of America, National Association of Science Writers, Sigma Delta Epsilon, Society for the Study of Evolution, Society of the Sigma Xi.

Bradford Hotel: Sections on Anthropology (H), Psychology (I), and Education (Q); American Nature Study Society, National Association of Biology Teachers, National Science Teachers Association, Pi Lambda Theta.

Commander Hotel: Sections on Astronomy (D) and Geology and Geography (E); American Astronomical Society, American Meteorological Society. Meetings of these sections and societies will be held at Harvard University.

Copley Plaza Hotel: Sections on Agriculture (O) and Botanical Sciences (G); American Fern Society, American Society for Horticultural Science, American Society of Plant Physiologists, American Society of Plant Taxonomists, Botanical Society of America, Mycological Society of America, Phi Sigma Biological Society, Potato Association of America, Sullivant Moss Society.

Kenmore Hotel: Sections on Physics (B), Chemistry (C), Social and Economic Sciences (K), History and Philosophy of Science (L), and Engineering (M).

Hotels adjacent to the Bradford are the Avery and Touraine; those adjacent to the Copley Plaza are the Charlesgate, Fensgate, Pioneer (for women), Copley Square, Lenox, and Vendome; those adjacent to the Kenmore are the Puritan, Braemore, Myles Standish, Sheraton, Buckminster, Gardner, and Minerva.

The Lincolnshire, Commonwealth, Bellevue, and Parker House hotels are grouped about the Boston Common and are within convenient walking distance of the Statler and Bradford hotels. The Commander and Continental hotels are adjacent to Harvard University.

Technical Papers

Effect of Dilution on Fertilizing Capacity of Rabbit Spermatozoa [1]

M. C. Chang

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In the determination of the minimal number of spermatozoa required to fertilize rabbit ova, Walton (*5*) suspended spermatozoa in 0.9 per cent of NaCl and inseminated 3 ml. of suspension into the vagina. Rowlands (*4*) used Baker's solution but inseminated 2 ml. According to them, 1,000,000 or more spermatozoa are required for maximum fertility. However, the writer (*1*) observed maximum fertility in 11 does following insemination of 1 ml. of 0.9 per cent NaCl, containing 330,000–420,000 spermatozoa.

Considering the fact that the motility and respiration of spermatozoa are very low in a very dilute sperm suspension (*2*), it was thought that the discrepancy of results might be due to the effect of dilution.

Twenty doe rabbits were superovulated according to Pincus (*3*) and inseminated with a known number or a similar number of spermatozoa suspended in 1, 0.4, or 0.1 ml. of saline. The does were killed 38 to 42 hours after insemination. The ova were flushed out and the number of cleaved ova were counted. The results show that 17–42 per cent, 0–28 per cent, and 0–6 per cent of the ova were cleaved when a similar number of spermatozoa (30,000–44,000) was suspended in 0.1, 0.4, and 1 ml. of saline, respectively. The mean per cent of cleavage (2.75) with the 1-ml. insemination is significantly different from the mean per cent (27) with the 0.1-ml. insemination. A maxi-

[1] The writer wishes to acknowledge his gratitude to Drs. G. Pincus and N. Werthessen for their encouragement. This work has been aided by a grant from the Foundation for Applied Research, San Antonio, Texas.

mum of 19 per cent of the ova cleaved when the number of spermatozoa was doubled (80,000) but suspended in 1 ml. of saline.

The advantage of a small volume of concentrated sperm suspension is clearly shown. It may be due to: (1) the detrimental effect of chemicals, ions, oxygen tension, etc. on spermatozoa when the proportion of chemical constituents in the medium to living tissue is excessive; (2) the fact that there might be beneficial chemical substances in the semen or in the spermatozoa which would be diluted too much in a large volume of solution with a consequent loss of fertilizing capacity; (3) the fact that the cervix can take up only a small amount of fluid and hence more sperms are taken up in a small volume of fluid. In any event, it is quite conclusive that the chance of fertilization is better when spermatozoa are suspended in a small volume of fluid.

Two implications arise from these findings: (1) In the diagnosis of male infertility, we have to take into consideration the volume of semen in relation to number of spermatozoa; that is, considering only the total number of spermatozoa in an ejaculate is not adequate for ascertaining the fertility of a male animal. It is the concentration of spermatozoa in semen that is more important. (2) In the practice of artificial insemination it is better to instill a small volume of fluid with a high concentration of sperms rather than a large volume of fluid with a low concentration of spermatozoa.

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Rapid Production of Acute Disseminated Encephalomyelitis in Rhesus Monkeys by Injection of Brain Tissue With Adjuvants[1]

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The production of multiple lesions of the central nervous system in monkeys by the repeated intramuscular injection of emulsions and extracts of rabbit brain has been reported by several investigators (*2, 11, 12*). The abnormal changes were marked by their wide dissemination, perivascular position, inflammation, proliferation of histiocytes, giant cell formation, and the associated demyelination. In all instances, large numbers of injections (30–100) and time intervals of from 3 to 13 months were required to induce the appearance of symptoms. Since this phenomenon may involve an immunological response to the injected brain material and the combination of the antibrain antibodies with the brain tissue of the animal to produce these pathological changes, it was thought that a more rapid effect might be obtained by the administration of brain tissue together with adjuvants. This procedure has been shown to result in an enhanced immune response with a variety of other substances (*1, 3–10*).

Two groups of four monkeys each were used. One group received an emulsion of 18 grams of rabbit brain in 20 ml. of saline, 20 ml. of "aquaphor," and 40 ml. of paraffin oil containing 95 mg. of dried, heat-killed tubercle bacilli (cf. *4*). The second group was given an emulsion of 27 grams of rabbit lung prepared in a similar manner. Both brain and lung materials contained phenol in a final concentration of 0.25 per cent and were heated to 60° C. for 45 minutes to destroy autolytic enzymes. Each monkey received three intramuscular injections of 1 ml. of material into the arm or leg at weekly intervals.

Three of the four monkeys that had received inoculations of brain tissue became ill from 25 to 33 days after the first inoculation or 9 to 19 days after the last injection. At first the animals were quieter than they had been before, sat hunched over, and were inadequately responsive to all stimuli. Shortly thereafter, focal signs of damage to the central nervous system appeared and grew rapidly worse. The localization, sequence of appearance, and speed of development of the signs varied in all three animals. Two showed marked trunk ataxia, and all showed some degree of weakness in one or more limbs. Rotation and retraction of the head and ptosis of the upper eyelids were noted in two animals, and a left internal strabismus and left facial weakness in one. Coarse muscular twitches were seen in all the limbs in one instance, and in another there was evidence of considerable reduction of vision.

The three affected monkeys became ill and were sacrificed by exsanguination on the day of the appearance of symptoms, and 2 and 8 days thereafter, respectively. In each instance it seemed that the animal might not survive for a longer period. Blood cultures proved sterile. Culture of cerebral tissue from one of the monkeys and intracerebral and intraperitoneal inoculation of a brain suspension into three mice and a rabbit were negative.

Post-mortem examination revealed lesions limited almost exclusively to the central nervous system. These resembled in all essential respects the changes

[1] Aided by grants from the William J. Matheson Commission.

produced by others (*2, 11, 12*) over a longer period of time. The lesions were focal, vascular, and perivascular and were most marked in the brain stem, particularly in the pons, and in the cerebellum. In the cerebrum they were widespread but fewer and generally less intense; the spinal cord was least affected. There was a marked predilection for white matter with some tendency to periventricular clustering. Involvement of contiguous grey matter, as well as independent lesions in the latter, were common but far less intense and frequent. In two instances moderately severe lesions were present in the optic nerves.

The pathological changes were characteristically in the walls of and about capillaries, venules, and veins, but arterioles and small arteries were not spared. An initial mural and perivascular infiltration by polymorphonuclear leucocytes (and occasional eosinophiles) gave way to a lymphocytic infiltration and a marked multiplication and hypertrophy of local histiocytes apparently from the blood vessel walls. Giant cells were not encountered. Occasional blood vessels showed degeneration of their walls, fibrin impregnation, and rarely perivascular hemorrhages or fresh thrombosis. These last features seemed to be the result of an intensification of changes already in progress. Myelin degeneration began as myelin pallor about an affected vessel and often coalesced about a group of these. It went on in the longest surviving monkey (8 days) to complete breakdown in some lesions. There was a microglial proliferation with phagocyte formation and a mild astrocytosis in the perivascular lesions. The axones seemed well preserved and undiminished in numbers, although some loss of these structures could not be ruled out. It is possible that, had the monkeys survived for a longer period, degeneration of axones would have been encountered (cf. *2*).

No symptoms were observed in the fourth monkey injected with brain material nor were any abnormal signs noted in any of the animals which had received the emulsion of lung tissue.

The fact that adjuvants enhance the effect of brain tissue in producing this pathological process supports the hypothesis that an antigen-antibody reaction is involved in the formation of these lesions. This is also indicated on histological grounds by the abundant histiocytic response and is indirectly corroborated by the absence of lesions in other organs; its specificity for brain is evidenced by the failure to produce the same results with lung tissue.

Summary. Rapid production in the monkey of a pathological condition resembling acute disseminated encephalomyelitis, marked by demyelination, can be achieved by the use of adjuvants added to rabbit brain emulsions.

(Since this paper was submitted, I. M. Morgan has reported (*J. Bact.*, 1946, **51**, 53, abstract) obtaining similar results with monkey spinal cord plus adjuvants.)

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Plant Carbonic Anhydrase[1]

Richard Day and Jane Franklin

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This paper reports evidence for the existence of a substance in, or associated with, the green leaves of the common elderberry bush (*Sambucus canadensis*) which catalyzes the reaction, $H_2CO_3 \rightleftarrows H_2O + CO_2$. Its chemical properties are similar to, but not identical with, those of animal carbonic anhydrase. Neish (*7*) in 1938 discovered the presence of carbonic anhydrase-like activity in chloroplasts but did not attempt to isolate the catalyst or to describe it further. Stimulated by Neish's work, Mommaerts (*6*) searched for plant carbonic anhydrase but found no evidence for its existence. Similar negative results had been obtained earlier by Burr (*1*) and by Roughton (*8*). Proceeding from the assumption that this catalyst is absent, Burr argued that the first step in photosynthesis cannot be the hydration of carbon dioxide. His calculations indicated that the speed with which carbohydrate production proceeds is so great that if the reaction, $H_2O + CO_2 \rightarrow H_2CO_3$, is part of the process, a catalyst similar to carbonic anhydrase is required. Failing to find such a catalyst, he concluded that some other step must be the initial one in photosynthesis.

We have found such a catalyst in elderberry leaves but not in the leaves of some 15 other species growing in the vicinity of New York, including one of those tested by Neish (burdock). Perhaps the enzyme,

[1] In this work, which was supported by a grant from the John and Mary R. Markle Foundation, much assistance was received from Anne D. and Hans H. Zinsser, in collaboration with whom a more complete description of the enzyme will be published later. The authors wish to thank David E. Green and F. J. W. Roughton for many valuable suggestions.

though present, is difficult to demonstrate in most species. Even in the case of elderberry leaves the activity decreases rapidly after separation from the bush. Another possible explanation of its absence or irregular occurrence in other species is that the enzyme is actually not a functional part of the leaf but is associated with some pathological condition from which the elder is especially liable to suffer. We have, however, found activity in all the specimens tested including those picked from bushes apparently in excellent condition and from those obviously diseased. Bushes from five different counties in southern New York State were examined.

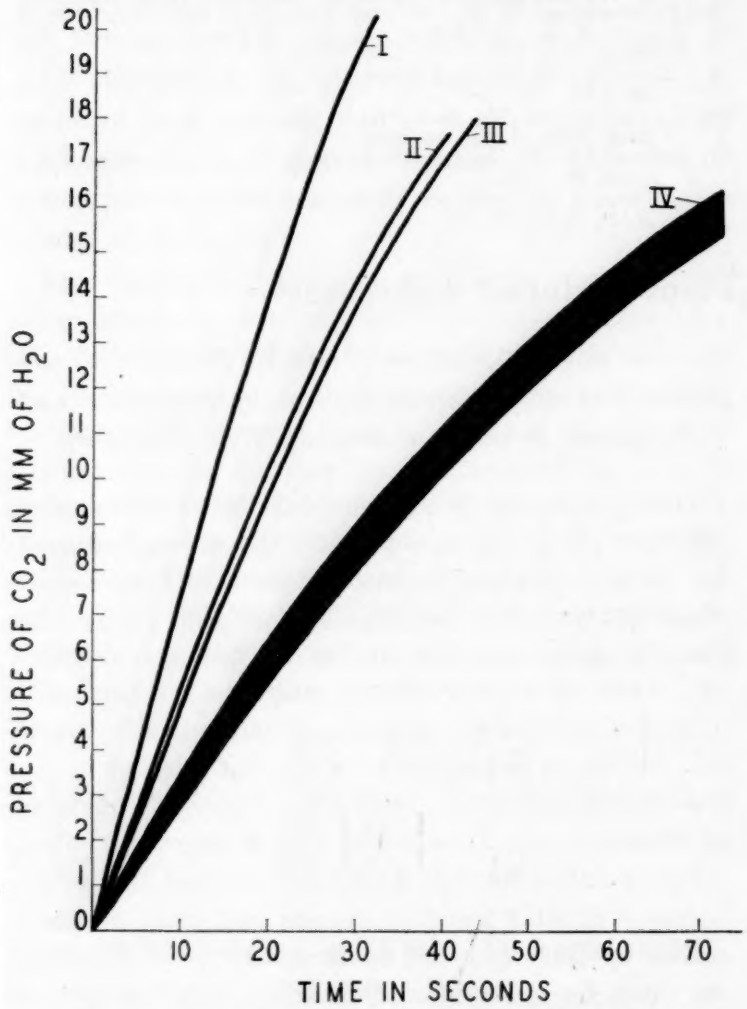

FIG. 1. Observations on the suspension. The curves indicate the rate of production of carbon dioxide under various circumstances when 2 cc. each of sodium bicarbonate solution and phosphate buffer of pH 6.8 are suddenly mixed at 15° C. (method of Meldrum and Roughton). For each curve a different substance is added to the substrate, as follows: I. 0.1 ml. of crude leaf suspension (curve identical to that obtained with added sulfanilamide); II. 0.1 ml. of 1 per cent laked human blood solution; III. 0.1 ml. of leaf suspension preserved 24 hours at 25° C. with 0.1 M stannous chloride; IV. Combined curve representing several curves which are so close together that separate identification is impossible. Included are three blank runs with no catalyst added and also observations with 0.1-ml. amounts of: (a) leaf suspension 24 hours old; (b) leaf suspension heated to 50° C. for 3 hours; (c) leaf suspension boiled 1 minute; (d) leaf suspension treated with 0.1 M $KMnO_4$; (e) suspensions of leaf substance of 15 different species of plants common to southern New York State.

Our original preparations were made by grinding a handful of leaves with sand and water for two minutes. The resultant green suspension was tested by the method of Meldrum and Roughton (*5*). Activity is expressed in units of E, an arbitrary velocity constant. In the test, 0.1 ml. of the green suspension is introduced into 4 ml. of substrate. This amount of crude suspension contains about 3 mg. of dried leaf; the resultant E is approximately the same as that obtained with 0.1 ml. of a 1-per cent solution of laked human blood containing approximately 0.2 mg. of solids. All the tests were made at 15° C.

The crude suspension is unstable. Activity is lost in 10 to 15 hours at 25° C. and in about 2 hours at 50° C. Boiling destroys all activity promptly. Activity is also destroyed by 0.001 M $KMnO_4$. Preservation of activity of the crude preparation can be achieved for several days by using 0.1 M $SnCl_2$ instead of water in making up the suspension. When the green particles, presumably chloroplasts, are separated by filtration or centrifugation, activity is entirely confined to the sediment, the clear supernatant being inactive (Fig. 1).

A solution, which is both clear and stable when stored at 10° C., was prepared by grinding the fresh leaves rendered brittle by contact with solid carbon dioxide and placing them in a cold, 25-per cent dextrose—0.1 M NaF solution. After a few hours all the leaf particles settled to the bottom, leaving a clear amber supernatant solution. The attempt to produce a stable dried powder failed. Leaves were ground in and desiccated by cold acetone. The resultant powder was highly active at first but deteriorated rapidly even when stored in the cold.

TABLE 1

INCREASE OF POTENCY WITH PURIFICATION

Specimen	Activity per unit of nitrogen expressed in arbitrary units				Average
	A	B	C	D	
Original solution of enzyme in 25% dextrose—0.1 M sodium fluoride	1.08	1.03	0.80	0.46	0.84
Sodium phosphate elution after adsorption on alumina-C-gamma gel	1.38	6.75	2.48	1.42	3.00
After both alumina gel treatment and precipitation with 50% saturated ammonium sulphate ...	8.35	7.75	4.42	2.91	5.86

The enzyme could be purified by ammonium sulphate fractionation between the limits of 40 and 60 per cent saturation. The activity ratio (ratio of enzyme units to protein concentration as determined spectroscopically by the absorption at 280 mμ) increased 3½-fold following ammonium sulphate precipitation and some 7-fold when ammonium sulphate fractionation was preceded by adsorption on, and elution from, alumina-C-gamma gel (Table 1). Since an

monium sulphate itself accelerates the test reaction, blank determinations with ammonium sulphate added to the reagents are necessary.

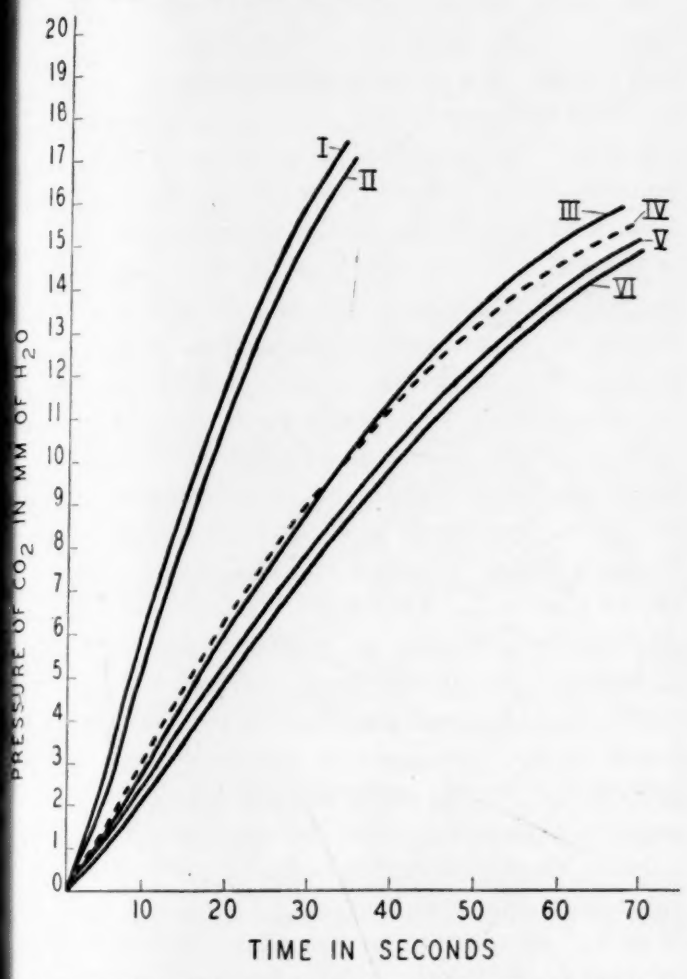

FIG. 2. Observations on plant enzyme solution: I. 0.1 ml. of dextrose-fluoride-enzyme solution after dialysis for 8 hours; II. 0.1 ml. of dextrose-fluoride-enzyme solution after storage for 4 weeks at 10° C.; III. 0.1 ml. of the same solution in 0.001 M KI; IV. 0.1 ml. of same solution as in II in 0.001 M $CuSO_4$; V. Same as II except heated at 80° for 15 minutes; VI. Blank. Curves I and II also are virtually identical to those obtained when the same solutions are used with sulfanilamide and sodium cyanide added.

The behavior of the active principle in the above-described procedures indicates that it is a protein. This opinion is supported by its lability to heat and by its failure to pass through a dialyzing membrane. A solution of the enzyme in sodium phosphate was dialyzed against 30 per cent ethanol for 8 hours. At the end of this time activity was unimpaired, but the concentration of phosphate had decreased to 2.5 per cent of the starting value.

The plant enzyme shows certain similarities to animal carbonic anhydrase but is different in other respects. Both are sharply inhibited by low concentrations (0.01–0.001 M) of H_2S, $KMnO_4$, $CuSO_4$, and I_2.

On the other hand, neither sulfanilamide nor cyanide appears to inhibit the plant enzyme, though both inhibit that obtained from animals (*4*) (Fig. 2).

Both the animal and the plant enzymes are soluble and stable in 30 per cent ethanol but are inactivated at concentrations of this alcohol above 40 per cent. In purified solution the plant enzyme is less heat labile than in the crude suspension; in fact, it is destroyed at the same temperature as the animal enzyme. Both are inactivated by 5 minutes at 80° C., at which temperature a precipitate forms. At 70° C. there is only slight inactivation even after half an hour.

Zinc, known to be a constituent of animal carbonic anhydrase (*3*), was found to be present in our solutions by the method of Hibbard (*2*). However, it cannot be stated whether this is associated with the active principle or with some other protein. Dialysis for 48 hours does not remove the zinc.

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News and Notes

About People

Irving Hill Blake has been appointed chairman of the Department of Zoology, University of Nebraska, to succeed David D. Whitney, who will remain on the staff as a professor of zoology.

L. W. Chubb, director of the Westinghouse Research Laboratories, is the recipient of the John Fritz medal and certificate, awarded annually for notable scientific or industrial achievement. The award is made by representatives of four national engineering societies: the American Society of Civil Engineers, American Institute of Mining and Metallurgical Engineers, American Society of Mechanical Engineers, and American Institute of Electrical Engineers.

Capt. Guy Wheeler Clark, USN, was appointed superintendent of the U. S. Naval Observatory, Washington, D. C., on 1 September, to succeed Capt. R. S. Wentworth, who has retired.

Howard F. Hunt has been appointed acting assistant professor of psychology at Stanford University, where he is connected with the general training program in

clinical psychology. Dr. Hunt will also be in charge of the Veterans Administration clinical training program.

Elmer Drew Merrill, Arnold professor of botany at Harvard University and former director of the Arnold Arboretum, has been honored by a special dedicatory number of the *Journal of the Arnold Arboretum* on the occasion of his 70th birthday on 15 October. This issue was edited by A. C. Smith and contains contributions from colleagues all over the world. Early in October a special issue of *Chronica Botanica,* entitled *Merrilleana* and consisting of a collection of reprints of Dr. Merrill's principal general writings, as well as a chronological biography and bibliography, was published in honor of Dr. Merrill.

Allan D. Maxwell, formerly acting chairman of the Department of Astronomy, University of Michigan, has joined the staff of the Nautical Almanac Office, U. S. Naval Observatory, Washington, D. C.

Donald C. Lowrie, director of the Museum of the Academy of Science of St. Louis, has been appointed professor of zoology at New Mexico Highlands University, Las Vegas.

Harry A. Allard, co-discoverer of photoperiodism, retired on 1 October after 40 years in the U. S. Department of Agriculture. In 1920 he and W. W. Garner announced in the *Journal of Agricultural Research* the principle that the blossoming and fruiting of plants depends upon the length of day. Knowledge of this principle has borne results in commercial horticulture and has stimulated increasing research in the field of photoperiodism which is today yielding useful facts. In addition to his pioneering work on the effects of light and dark periods on plants, Mr. Allard did early work in the investigation of tobacco mosaic disease and in scientific tobacco breeding. The record of his published papers covers nearly 50 years.

Norman R. Davidson, recently of the RCA Laboratories, Princeton, has been appointed an instructor in general chemistry at the California Institute of Technology, according to an announcement by Linus Pauling.

Announcements

Leading librarians and museum directors of the country will be honored on 19 October at a convocation culminating a two-day program in celebration of the return of Yale University's collections to peacetime use. Several receptions have been planned to open special exhibitions.

On the afternoon of 18 October the unveiling of a natural history mural and several dioramas will be held in the Peabody Museum of Natural History. The mural, which shows the great dinosaurs of 200,000,000 years ago in their natural habitat, was painted by Rudolph F. Zallinger, of the Yale Fine Arts Faculty, and covers the east wall of the Hall of Dinosaurs. The dioramas, showing some of Connecticut's plants and animals in a series of selected environments, were the work of Perry Wilson, on leave from the American Museum. Also on display at the same time will be paleontological collections made by Marsh, Beecher, Schuchert, and Wieland; the minerals, obtained early in the 19th Century, which established Yale's eminence in geological studies; and the zoological collections formed by Verrill and Petrunkevitch. Representative groupings from the University's 1,250-item anthropological collection will also be on display. Best known of these are the Marsh and Grinnell Collections of the American Plains Indians and the Peruvian finds made and given by former U. S. Senator Hiram Bingham. Friday's formal program will end with a reception in the evening in the Art School, when William M. Ivins, Jr., curator emeritus of prints in the Metropolitan Museum of Art, will give an address. The third reception, which opens an exhibition of 300 noteworthy books in the Yale Library, will be held on Saturday afternoon. In this exhibition Sir Isaac Newton's own copy of *Principia,* which he gave the Yale College Library shortly after its founding, will be of special interest to scientists.

At the convocation on Saturday evening, the degree of Doctor of Science will be conferred upon: Alfred L. Kroeber, director emeritus of the Anthropological Museum, University of California; Albert Eide Parr, director of the American Museum of Natural History, New York City; and George Gaylord Simpson, curator of the paleontological collections, American Museum of Natural History.

Among the librarians to receive degrees are Luther Harris Evans, Harry Miller Lydenberg, Keyes DeWitt Metcalf, and Lawrence Counselman Wroth.

The Jesup Lectures, given under the auspices of the Department of Zoology, Columbia University, are being delivered this year by G. Ledyard Stebbins, Jr., of the University of California, Berkeley, on "Variation and Evolution in Plants." The series of lectures opened on 15 October. Subsequent lectures will be given on 22 October, 29 October, 12 November, 19 November, and 26 November at 5:00 P.M. in Columbia's Schermerhorn Hall.

Three emergency colleges for veterans have been organized for freshman and sophomore curricula by New York State. These colleges—Sampson at Sampson, Mohawk at Utica, and Champlain at Plattsburg—are being converted from former military installations at these locations.

The appropriation for the colleges and the operating corporation, named the Associated Colleges of Upper New York, were created by act of legislature last March. The Board of Trustees consists of the presidents of New York State colleges and universities, including Clarkson, Colgate, Cornell, Colleges of the Seneca, Hamilton, Rensselaer Polytechnic Institute, Rochester, St. Lawrence, Syracuse, and Union. The president chosen by the Board is Asa S. Knowles, formerly of Rhode Island State College.

The faculties of the colleges, two of which have opened within the last month, have been chosen from among retired teaching personnel, persons recently released from the services or government agencies with teaching experience, graduate schools and placement bureaus of colleges and universities throughout the country, the professional division of the U. S. Employment Service, and the U. S. Department of Labor's National Roster of Scientific and Specialized Personnel.

The curriculum, including engineering, liberal arts, and business administration, has been especially designed for freshman and sophomore students, who may transfer elsewhere for their third and fourth years of academic work. Full credit will be given by established schools for the courses completed at the emergency colleges. Extracurricular activities, such as intercollegiate and intramural sports programs, will also be provided.

Over 9,000 inquiries have been made by veterans and others from New York and other states, and about 2,000 completed applications have been accepted. The colleges will have accommodations for 6,800 students this fall with an ultimate capacity of more than 10,000. Registration for the three institutions will not be limited to male veterans. At present, however, only the wives of students will be permitted residence on the campus, all others attending on the day school basis.

A series of lectures on physicochemical methods and their applications in research and technology will be presented by specialists of Mellon Institute during 1946–47. The lectures will be delivered on Thursdays at 11:40 A.M., both semesters, in the auditorium of the Institute. The series is open to all students in the professional courses in chemistry and chemical engineering in the University of Pittsburgh, to the Institute's members, and to interested persons in the district.

The following sessions have been scheduled: J. R. Bowman: "Molecular Distillation," 24 October; J. R. Anderson: "Ebulliometry," 14 November; G. C. Akerlof: "Analytical Instruments," 21 November; H. P. Klug: "Electron Optics," 12 December; A. L. Marston: "Spectroscopy," 9 January; L. E. Alexander: "Crystal Structure," 23 January; E. P. Barrett: "Sorption," 20 February; W. M. Kutz: "Catalysis," 6 March; R. H. Hartigan: "Synthetic Methods," 20 March; T. W. DeWitt: "High-Vacuum Techniques," 10 April; J. T. Kummer: "Isotopes and Tracer Techniques," 24 April; and J. W. Jordan: "Colloid Techniques," 8 May.

Karl T. Compton told of recent changes at M.I.T. in his address, "On Engineering Education," delivered at the Princeton Bicentennial on 4 October. Among these changes are: the addition of a new engineering department, Food Technology; simplification of the undergraduate curriculum; and an increase in the proportion of graduate students. Courses in the fields of humanities and social sciences have been intensified. New subjects, such as the gas turbine and jet propulsion, supersonic air flow, and nuclear chemistry, have been introduced, and previous subjects, such as instrumentation, servomechanisms, electronics, nuclear physics, applied mathematics, and acoustics, have been substantially strengthened.

A group of Centers of Research have been set up under interdepartmental auspices to handle important fields of work which extend across the conventional departmental boundaries. Among these Centers are: the Research Laboratory of Electronics, the Research Laboratory of Nuclear Science and Engineering; the Research Laboratory of Acoustics; the Spectroscopy Laboratory; and the Center of Analysis.

Attempts are being made to derive the greatest educational benefits for the senior, postgraduate, and postdoctorate levels through sponsored research, handled on behalf of industry and governmental agencies through the Institute's Division of Industrial Cooperation.

Meetings

The Eastern Association of Electroencephalographers will hold their fourth meeting on 26 October at the Yale School of Medicine, New Haven, Connecticut. The Scientific Meeting, scheduled for 2:30 P.M., will include the following lectures: Margaret Lennox: "Some Effects of Deep Brain Lesions on the Electroencephalogram"; Frederick Redlich: "Some Other Uses of the Electroencephalograph"; and Mary A. B. Brazier: "A Review of the Electroencephalographic Findings at Various Levels of O_2 and CO_2." Leslie F. Nims will act as chairman for the discussions.

The American Society of Agronomy and *The Soil Science Society of America* will hold their annual meetings on 19–22 November at the Hotel Fontenelle, Omaha, Nebraska.

The Sixth International Congress on Experimental Cell Research will be held in Stockholm in July 1947. The Congress will be organized by a Swedish working committee. J. Runnström, of Wenner-Grens Institute, will act as chairman for the conference, and T. Caspersson and H. Hyden, of the Karolinska Institute, as secretaries. A preliminary program will be published this fall. The conference will include a series of symposia on important problems in experimental cell research from physicochemical, physiological, and morphological aspects. The Swedish organizing committee hopes that cell research workers in all fields will take advantage of this occasion for exchanging and renewing contact with their colleagues. Suggestions or questions regarding the conference should be sent to the secretaries.

Recent Deaths

Robert Lemuel Sackett, 78, dean emeritus of the School of Engineering, Pennsylvania State College, died on 6 October in New York City. Dr. Sackett was a former vice-president of Section M, AAAS.

John E. Walter, 29, assistant professor of physics, Princeton University, died on 23 September in Princeton, New Jersey.

H. C. Plummer, 70, fellow of the Royal Society (England), died on 30 September at his home in Oxford. Dr. Plummer's most well-known work was *An introductory treatise on dynamical astronomy.*

H. L. Cooke, 67, since 1919 professor of physics at Princeton University, died on 30 September in Princeton, New Jersey.

Support for Animal Experimentation

Under the date of 12 August, Howard Strong, secretary of the Health and Advisory Council of the U. S. Chamber of Commerce, reported to A. J. Carlson that a poll of the member organizations resulted in an overwhelming majority in favor of the following resolution:

> In view of the great progress that has been made in preventive and curative medicine and surgery through animal research and the prospect of even greater progress in the future, the National Chamber is unalterably opposed to the prohibition of this scientific procedure. Such a prohibition would seriously hamper all medical progress.

A total of 2,424 member organizations approved of the statement, and 18 organizations did not approve. Since these organizations have approximately 1,000,000 members from the business and professional fields, the support is tangible. Furthermore, the U. S. Chamber of Commerce is in a position to present its opposition to antivivisection legislation wherever it might appear, and, when advisable and possible, a representative of the Chamber can actually appear in opposition to any such measure.

The secretary's report acknowledged the influence of Dr. Carlson in bringing about the adoption of the resolution.

A second resolution in favor of animal experimentation was unanimously passed by the membership of the American Diabetes Association in session on 17 September at Toronto:

> WHEREAS, the American Diabetes Association at this meeting is commemorating the 25th anniversary of the discovery of insulin; and whereas, insulin has been instrumental in restoring the health and saving the lives of countless human beings suffering from diabetes; and whereas, the great work of Banting and Best in discovering insulin, and the subsequent scientific investigations clarifying its actions and uses, would have been impossible without the use of dogs and other domestic animals as experimental subjects; therefore, be it resolved that the American Diabetes Association hereby testifies to the value of the use of dogs and other domestic animals for purposes of scientific research and urges all enlightened citizens to refrain from supporting the misguided efforts of so-called antivivisectionists, who constantly try to hamper the advancement of scientific medicine.

The National Science Foundation and Military-sponsored Research

Otto Stern, professor emeritus of physics, Carnegie Institute of Technology, and Nobel Laureate in Physics (1943), speaking for the Executive Committee of the Northern California Association of Scientists, has recently called attention to the dangers in the present broad-scale sponsoring of university research by military agencies and pointed out the necessity for a speedy enactment of a National Science Foundation bill. The statement says:

> The Army and Navy are outstanding examples, and it is highly commendable that, recognizing the value of fundamental research, they have assigned extensive funds to universities, under liberal contracts which permit research relatively free of restrictions. But the fact that scientists must depend on the Army and Navy for their funds may ultimately defeat the purpose for which these funds are provided. In raising this criticism it should be made clear that it is not the Army and Navy who are at fault: they have acted most intelligently in recognizing the need for a more adequate support of basic research; this criticism is directed at the American Congress for its delay in passing the bill establishing a National Science Foundation.
>
> The reason for the undesirability of military sponsoring of research lies in the nature of fundamental research and in the atmosphere necessary for its pursuit. Fundamental research consists of the discovery and elucidation of natural phenomena and the subsequent formulation of

the basic laws of nature. Very often such discoveries and laws have no immediate practical applications, serving only as a contribution to the general framework of theory which is a fundamental part of science. History teaches us that these researches have often the greatest significance in the light of later discoveries. A striking example is the development of the practical utilization of atomic energy, which depended on chemical and physical knowledge dating back many years in scientific history, extended by individual investigators in many lands. For this kind of research a certain atmosphere is required. The scientist must be free to exercise the full range of his curiosity and imagination, without having to justify his experiments for the practical minded, or to seek practical application of his theories, or to write monthly reports. For its most fruitful fulfillment, fundamental research must be completely divorced from concern with practical matters of this sort. Grants for fundamental research should be made at the discretion of a civilian board made up of scientists qualified to judge the value of a research project, and above all the researcher. Such a decision should not reside in a military or political officer, nor should a scientist be accountable for his work to a military or political officer.

Only under conditions of completely free fundamental research will the best possible development of science continue. It is fortunate that funds have become available for the more extensive support of university research; the need for such funds is great. But it should be recognized that Army and Navy sponsoring of research is only a stop-gap arrangement, serving until the time that a National Science Foundation is established by the Federal Government. We therefore urge the speedy enactment of a bill establishing a National Science Foundation.

Dr. Stern is now living in Berkeley at 759 Cragmont Avenue.

War Department Research and Development Division

On 19 September the War Department announced a civilian appointment of special interest to scientists. Pres. Cloyd H. Marvin, of George Washington University, will be the new Deputy for Research.

The Research and Development Division was organized this spring under the direction of Maj. Gen. Henry S. Aurand, who is now adviser to the Secretary of War and the Chief of Staff on all War Department matters relating to both research and development.

Gen. Aurand's Deputy for Development is Brig. Gen. Earl S. Hoag, who was a wing commander in the Air Transport Command during World War II. Gen. Aurand was the commanding general of the Sixth Service Command from September 1942 to October 1944. Since 1944 he has had several commands overseas and most recently has returned as commander of Service of Supply in the Chinese Theater.

Dr. Marvin, who will continue as president of George Washington University, has recently set up an office in the Pentagon building, where he will be supported by a staff of scientists who will represent primarily the broad fields of science and engineering which impinge most directly on War Department research interests.

The Division will also include representatives of the social science fields on its scientific staff to attack the broad problems of utilization of rare and highly trained man power.

These scientists will maintain the closest contact with research and development activities in War Department laboratories. In addition, they will work closely with other Services and with the research programs prosecuted by civilian institutions throughout the United States.

Their work will be supplemented by a corps of technically trained Army officers, and a panel of consultants drawn from the highest levels of science, education, and industry will stand ready to assist with expert counsel whenever needed.

Since the War Department realizes that the permanent withdrawal of scientists from fundamental research weakens the Nation's scientific future, these scientific advisers will be asked to serve for only one year on a rotational basis. The cooperation of the various scientific societies is being sought to maintain this rotation. Scientists who have worked with the Division will be asked to serve on the panel of consultants so that their experience can be further utilized. There will be created in this way a group of scientists familiar with War Department needs and problems, to supplement those who aided so materially in military research during World War II.

Dr. Marvin's knowledge of the War Department dates back to World War I, when he served as a captain in the Army Aviation Service. Subsequently he became dean at the University of California, then president of the University of Arizona, and went to George Washington University as president in 1927.

Gen. Aurand has devised a pattern of close integration between research, on the one hand, and development, on the other, within the War Department. But the role of the new Division will be not only that of coordinating War Department research. It will insure the closest cooperation between military and civilian organizations in attacking the grave problems which must be overcome to keep the United States in the forefront of military research. Gen. Aurand is firmly committed to this policy of cooperation and holds strongly that future planning for defense must involve the concept of a steady and enduring partnership between science, industry, and the military organization.

In the Laboratory

The Differentiation of Penicillins G and K by an Assay Method *in Vivo*

MARGARET BUCK, ANNETTE C. FARR, and R. J. SCHNITZER

Chemotherapy Laboratory, Hoffmann-La Roche, Inc. Nutley, New Jersey

The activity of penicillin toward experimental infections of mice with Borrelia formed the basis for a rapid *in vivo* assay method of penicillin used in this laboratory during the last two years.

It was found that a single subcutaneous injection of 15,000 or 25,000 units penicillin G/kg. (dependent on the strain of Borrelia used) administered to mice in the active multiplication phase of the spirochetes in the blood decreased the number of spirochetes gradually, until almost all parasites had disappeared within 3 hours. This reduction of parasites remained constant for at least 20 hours; the ratio of the 3-hour value to the 20-hour value was 1.0 or not higher than 1.1.

Recent observations by Eagle and Musselman (*2*) and Coghill, Osterberg, and Hazel (*1*) showed that penicillin K was considerably less active *in vivo* (rabbit syphilis, streptococcal and pneumococcal infections) than penicillin G and produced very low blood levels. In experimental Borrelia infections of mice penicillin K[1] was found to be much less active than penicillin G: subcutaneous treatment with doses of 15,000–50,000 units/kg. was without effect; a temporary reduction of the number of parasites in the blood was observed after administration of 100,000 units/kg.

These findings seemed to indicate that the *in vivo* assay with Borrelia might be useful for the determination of penicillin K in mixtures.[2]

Experiments were carried out with artificial mixtures of crystalline penicillin G sodium salt (supplied by M. W. Goldberg and W. E. Scott from the Roche Chemical Research Laboratories) and crystalline penicillin K (about 90 per cent pure K) prepared on a weight basis. The unitage of the mixtures was calculated on the basis of 1,666 units/mg. G and 2,000 units/mg. K and then checked by the cup test method. The figures for the potency *in vitro,* for which we are indebted to B. Tabenkin and G. Fels, were in close agreement with the calculated values, showing an occasional variation of not more than 5 per cent.

Tests were carried out with a strain of Borrelia isolated from *Ornithodorus turicata* in this laboratory about four years ago and with the laboratory strain of *Borrelia novyi* (kindly supplied by Q. M. Geiman, of Harvard Medical School). The latter strain was somewhat less sensitive toward penicillin than the younger strain isolated from ticks but produced consistently more severe infections. Only the results with this strain are presented.

PROCEDURE

Albino mice of 16–21 grams were infected intra-abdominally with 0.5 cc. of a suspension of mouse blood containing 8–10 parasites/microscope field (dark-field illumination; approx. 600 lin. magnification), corresponding to approximately 30,000 parasites/mm^3.

The infected animals showed a well-developed infection of the peripheral blood after 22–24 hours. The initial count was 700–900 parasites/100 fields. Infections with less than 400 or more than 1,500 parasites/100 fields seemed to be less suitable for this type of experiment.

The animals, divided into groups of five, received a single subcutaneous injection of 25,000 units/kg., using the solutions of the pure penicillins or their mixtures.

Counts were originally taken every hour up to 3 hours and after 20 hours. Since experience indicated that the decisive values were obtained 3 hours and 20 hours after the treatment, we confined ourselves to determining the number of parasites at these two intervals. The actual counting was done by dark-field microscopy. In cases of heavy infections it seemed sufficient to count 10–20 fields, but if the number of parasites was very small, 60–100 fields had to be examined.

RESULTS

The results as given in Table 1 are based, without exception, on at least six but generally more (up to 20) experiments. Due to the scarcity of penicillin K at our disposal fewer experiments were performed in which larger doses of K were required.

The figures representing per cent reduction from the initial count show that the activity of penicillin G was

[1] We are indebted to Charles Pfizer and Company for the penicillin K employed.

[2] In a recent publication by J. Williamson and E. M. Lourie (*Brit. med. J.,* 1946, 828–829) evidence is brought forth that penicillin III (= X) is also less active in Borrelia infections than penicillins F and G.

very consistent and produced an almost complete reduction of the number of parasites for 20 hours. Penicillin K, on the other hand, was of very low activity even if a four times higher dose was given. The presence of 30 per cent penicillin K in mixtures of G and K was evident not only by the lower initial reduction but especially by the increase of parasites after 20 hours. The ratio t 3 : t 20 was in all instances greater than 1.1. If 50 per cent K were present in the mixtures, a still greater drop in activity was observed. In the untreated controls the number of parasites increased steadily and was generally 50–100 per cent higher after 3 hours and three to five times higher than the initial count after 20 hours. Similar results were obtained with the other Borrelia strain.

TABLE 1

ASSAY OF PENICILLINS G AND K AND MIXTURES OF G AND K IN *B. novyi* INFECTIONS OF MICE
(Initial count/100 fields: 798 ± 160; single subcutaneous treatment)

Penicillin		Dose units/kg.	Reduction from initial parasite count (%)		Ratio t 3 : t 20
% G	% K		t 3*	t 20†	
100	0	25,000	98.7 ± 1.8	92.5 ± 3.6	1.06
70	30	25,000	93.4 ± 5.3	75.6 ± 9.2	1.22
50	50	25,000	84.3 ± 6.9	46.0 ± 2.3	1.83
0	90	100,000	76.0 ± 3.7	53.7 ± 11.8	1.43

* 3-hour interval.
† 20-hour interval.

From these experiments the conclusion was drawn that 25,000 units/kg. of a penicillin containing more than 70 per cent penicillin G would reduce the initial number of *B. novyi* by not less than 95 per cent (usually more) within 3 hours, the reduction lasting 20 hours.

Although there are indications that this *in vivo* assay technic might be developed to a method of higher sensitivity, it seems that approximately 30 per cent K could be determined in a mixture of active penicillins with the present procedure.

Routine assays with penicillin mixtures from production batches demonstrated that the Borrelia test carried out with crystalline G and an artificial mixture of 70 per cent G and 30 per cent K as standards was sufficiently sensitive for practical purposes, e.g. for the study of the influence of precursors.

Whether the *in vivo* assay will be preferable to the differential assay with *Bacillus subtilis* R and *Staphylococcus aureus* (*3*) for the purpose of production control cannot yet be decided. In case of artificial mixtures there was a fairly good agreement of the *in vivo* and *in vivo* determinations.

The question arose whether other ***in vivo*** assay methods, e.g. with bacterial infections, could be used for the determination of penicillin K. The low activity of penicillin K in pneumococcal and streptococcal infections as demonstrated by Eagle and Musselman (*2*) seemed to suggest such a possibility. It may be seen from Table 2 that the presence of 50 per cent K in an artificial mixture of pure penicillins could easily be detected; the presence of 30 per cent K did, however, not interfere significantly with the antibacterial activity.

TABLE 2

ACTIVITY OF PENICILLINS G AND K AND THEIR MIXTURES IN EXPERIMENTAL INFECTIONS OF MICE WITH 1,000 MLD OF TYPE 1 PNEUMOCOCCI (STRAIN 6301) AND β-HEMOLYTIC STREPTOCOCCI (STRAIN #4)

Penicillin		Total dose units/kg.	Organism	Number of mice survivors		Survivors (%)
% G	% K					
100	0	3,000	type 1 pneumococci	20	14	70
75–70	25–30	3,000		10	9	90
50	50	3,000		10	2	20
25	75	3,000		10	3	30
0	90	6,000		10	1	10
0	90	12,000		10	4	40
..	..	Controls		20	0	0
100	0	1,000	β-hemolytic streptococci	10	9	90
70	30	1,000		10	7	70
50	50	1,000		10	4	40
..	..	Controls		10	0	0

Even if it would be possible to increase the sensitivity of an *in vivo* assay method in bacterial infections sufficiently for the determination of smaller quantities of penicillin K, these methods would always require at least a five-day observation period before a definite result could be obtained.

The advantage of the Borrelia assay technic is that it requires no more time than the *in vitro* methods.

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Apparatus for the Prolonged Sterile Culture *in Vitro* of Whole Plants or Excised Plant Tissues

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An apparatus for prolonged sterile culture *in vitro* of whole plants or excised plant tissues should meet

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the following requirements: (1) The nutrient medium should be easily removable for renewal or analysis without disturbing the cultures; (2) adequate aeration of roots or isolated tissues should be afforded; and (3) the fluid medium should be kept in circulation to ensure an even supply of nutrient. These requisites can be met by the type of culture vessel shown in Fig. 1, used in conjunction with the apparatus shown in Fig. 2.

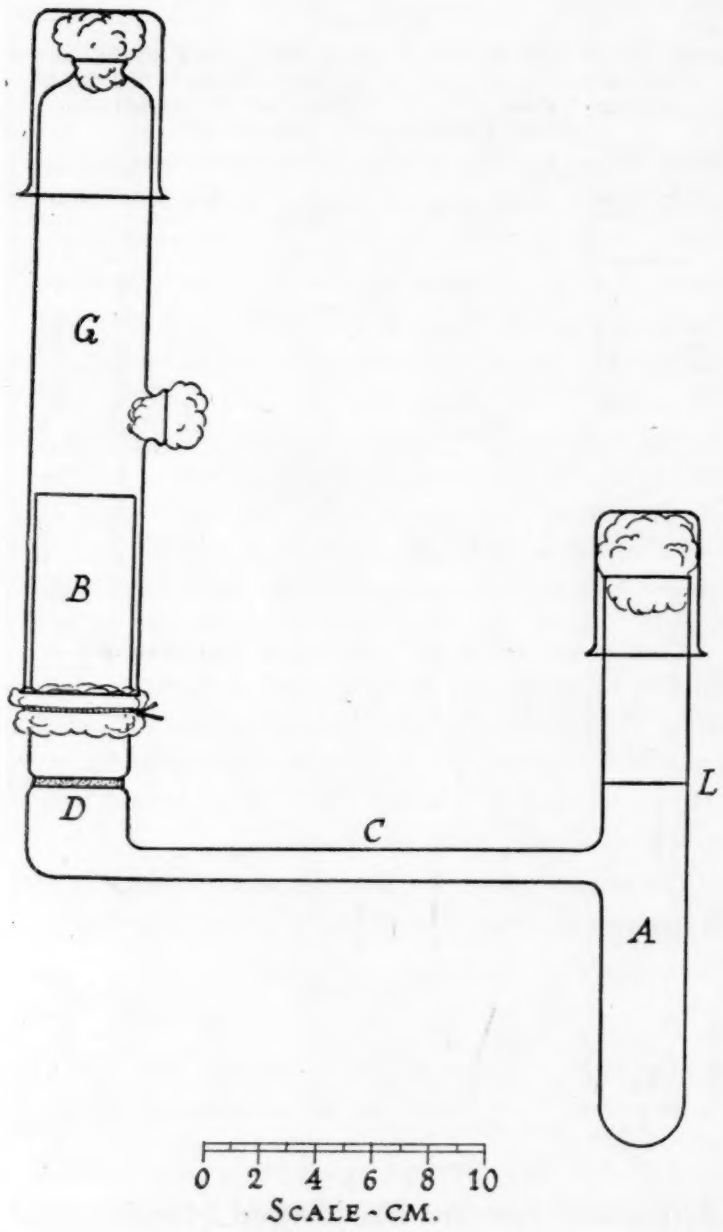

FIG. 1

The culture vessel consists of two main parts: the nutrient medium reservoir, A, and the growing chamber, B. These are connected by a sidearm tube, C. At the base of the growing chamber is a sintered glass disc, D, on top of which can be placed the substrate, a layer either of washed quartz sand or of small glass balls, depending upon the nature of the culture material. On this layer is placed the material to be cultured. The reservoir, A, is filled with sterile nutrient medium to level L, which ensures that, when the vessel is held upright, the medium in B reaches the level of the sintered glass disc. The liquid nutrient medium is brought into contact with culture material by slowly tilting the culture vessel so that the position of B is lowered while that of A is raised. As a result of this change of position, the fluid nutrient medium rises through the sintered glass disc to the desired level in the growing chamber. The rise of the fluid level in B can be adjusted either to immerse the material completely or merely to flood the substrate on which the tissue culture is placed. A detachable tube, G, is fitted over the open top of the growing chamber and rests on a layer of cotton, wrapped and tied securely around the growing chamber. The height of the tube, G, with the cotton wrapping supporting it, can be adjusted to allow increased space for growth increment

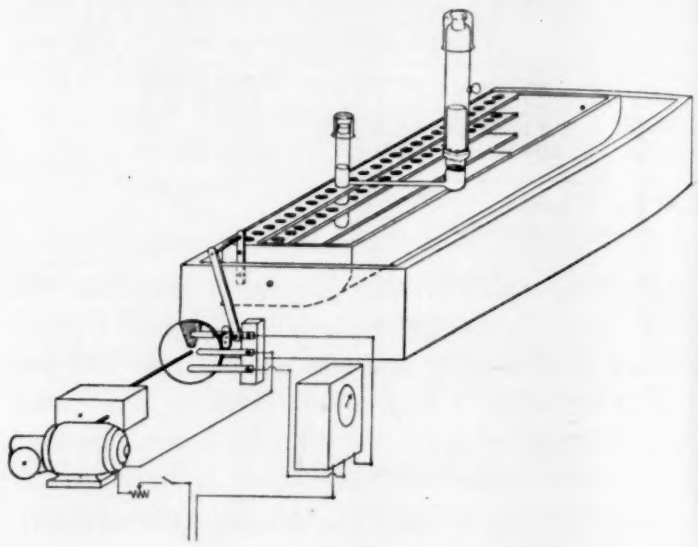

FIG. 2

of the culture. Side and top openings in the detachable tube make possible the manipulation of the culture material without exposing it to the atmosphere.

The apparatus presented in Fig. 2 consists of a rectangular wooden frame within which fits a rack attached to the frame by means of a pivot at each end. The A tube of the culture vessel is fitted into the rack which, when in the horizontal position, holds the culture vessel upright.

At one end of the frame and on a platform attached to it is secured the mechanism which furnishes the motion for tilting the rack. The power is supplied by an electric motor working through a reduction gear and attached, by a shaft, to a cam. A small and a large operating arm, joined by a rod, convert the rotating motion of the cam to a reciprocating motion which tilts the rack. The small operating arm is attached to the camshaft, and the large operating arm to a rod secured to the rack at one pivoted end. A time switch, which activates the electric motor, is also attached to the cam. An insulated strip inserted in the brass cam breaks the circuit to the motor when the rack has been tilted to the necessary angle. It

remains thus tilted until the motor is again activated, the circuit being broken by the insulated strip when the rack has returned to the horizontal position. By this means the culture material can be maintained in contact with the nutrient medium for any given period.

The size and form of the growing chamber, B, can be made to conform to the requirements of the material to be cultured. Entire plants, such as sunflowers, require a vessel 35 × 200 mm. in diameter, containing quartz sand to a depth of 170 mm. For the culture of small fragments of plant tissue the upper tube, G, can be dispensed with, and the fragments cultured in a tube 25 × 200 mm. Molds also can be cultured by this means, provided that the fluid medium level is so arranged as to ensure the maintenance of a layer of liquid between the mycelium and the sintered glass disc, which must be of sufficient fineness to prevent spores from being washed back into the nutrient medium reservoir. Optimum frequency of immersion must be determined separately for each type of tissue. In general, plant tissues grow most satisfactorily if their immersion is infrequent. One immersion of 5 minutes duration in a period of 24 hours was sufficient for sunflower stem tissue if supported on quartz sand. The nutrient medium can be changed as often as is desired by emptying vessel A with a sterile pipette and introducing fresh nutrient to level L.

A High-Capacity Sensitive Relay

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A high-capacity relay is often needed for the control of heating elements in constant-temperature cabinets and water baths that will operate on a minimum amount of current. The sensitivity of most thermostats is usually affected by the arcing at the contact points. Such a relay has been developed to reduce this arcing to a minimum.

By using a solenoid instead of an electromagnet it is possible to operate an arm bearing a mercury contact tube with a minimum amount of current. Mercury contact tubes are available with rated capacities of 5-35 amperes.

The assembled relay is diagramed in Fig. 1. The base for the relay was constructed from seasoned oak (K). The mounting for the solenoid was made from a brass plate (A). Four small holes were drilled in the base for roundheaded brass screws. The holes were made slightly larger than the screws in order to permit final adjustments in the position of the solenoid in relation to the arc described by the end of the arm. A hole ¼ inch in diameter, of the same size as the brass casing of the solenoid (B), was drilled in the center of the brass mounting plate, to which the solenoid casing was then soldered. The bearing support (F) for the mercury tube was made by cutting a rectangular brass bar into three pieces, two pieces 2 inches long for uprights and one 3 inches long for the base, and soldering them together to form a U.

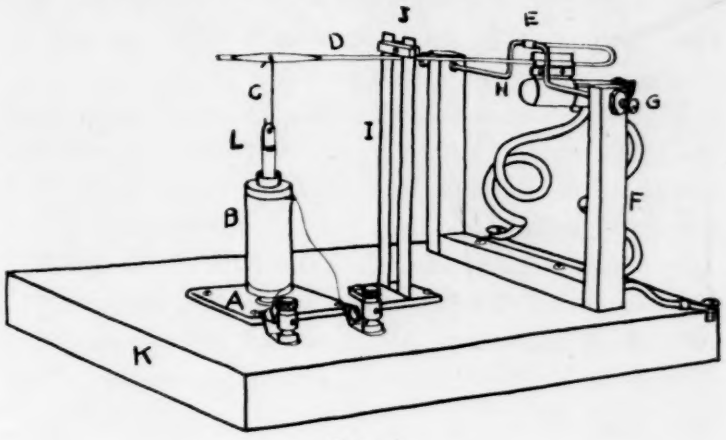

Fig. 1

It was necessary to make a wide support for the mercury tube to allow free movement of the connecting wire. Holes (G) ⅛ inch in diameter were drilled ½ inch from the top of the support and threaded with an 8–32 tap. Through these holes were screwed two ½-inch brass bolts (G), the ends of which were drilled to a depth of 1/16 inch to provide a bearing for the relay arm assembly. Each bearing was held in a fixed position by a hexagonal lock nut.

The arm (D) for the mercury tube was made from 12-gauge, galvanized-iron wire about 9 inches long and bent into the shape shown in the diagram. Both ends were flattened, one for drilling the hole for the link to suspend the soft-iron plunger and the other end to solder to E. A second piece of 12-gauge wire (E) was bent to form a U, 1 inch in width and height, with projections to be used as a shaft. The U wire was then soldered to the arm (D) 4 inches from the hole drilled for the iron plunger (L). The holder for the mercury tube was made from light-gauge tin plate. The mercury tube and holder (H) were then clamped to the arm by small machine bolts. This type of clamp provided a final adjustment of the instrument and made it possible to reverse the action of the tube for special requirements. The movement of the arm was controlled by means of an adjustable stop made of two small pieces of heavy brass (J) fastened with small machine bolts to two strips of brass (I) soldered to a brass base. The connecting link (C) for the soft-iron plunger was made from a piece of 16-gauge steel wire after assembly of the instrument. The soft-iron plunger was made from a piece of 6-gauge iron wire,

this size allowing about 1/32 inch on all sides for free movement of the plunger. One end was flattened to drill the hole for the connecting link. Binding posts were then attached to the leads from the solenoid and the mercury tube.

In the heating circuit the tube was operated as a single-pole, single-throw switch. The current for the solenoid was supplied by an ordinary 6–8 volt a-c bell-ringing transformer. A 0.25-microfarad condenser was used across the terminals of mercury thermostats (not shown). The final adjustment of the stop (J) was made while the instrument was in operation. A 5/16-inch movement of the plunger would make and break the circuit, but to insure positive action of the mercury tube the stop was set for a ½-inch arc. The mercury contact tube used in the construction of the relay was a General Electric KON-NEC-TOR, 40KRI. Other types of mercury tubes would give the same results with the relay, but it would be necessary to change the length of the arm, depending on the tilting action.

This relay has been used with open and closed types of mercury thermostats with no failures. The relay may be used with direct current (4–7.5 volts), but it will be necessary to determine the direction of the lines of force through the solenoid before soldering to the base.

A Punched-Card Technique for Computing Means, Standard Deviations, and the Product-Moment Correlation Coefficient and for Listing Scattergrams

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Punched-card techniques are not familiar to the general scientific public. While laboratory workers are devoting hours to tedious calculations, machine facilities frequently have completed monthly routine reports and are lying idle in the accounting department. But with sketchy information at the level of this note the laboratory man should be able to speak the language of the punched-card specialist well enough to arrange basic computations and tabulations. Although the specialist will know punched cards and in all probability will be able to clear up at once any problems in planning the card, his knowledge is likely to be deficient in the special applications of cards to statistical studies.

The sorting and tabulating operations to obtain means, standard deviations, the product-moment coefficient, and the scattergram are detailed step by step below. Only two points must be grasped to understand these operations. The first is that, after two consecutive sortings of data, the individual groupings resulting from the second sort are in order according to the first sort. The second point is the theory underlying the method for computing sums of squares and sums of cross-products by progressive additions. Details can be found elsewhere (*1, 3*), but they can be reconstructed readily if one recalls that multiplication may be accomplished by adding the multiplicand as many times as the multiplier.

Machine Sorting and Tabulating Operations After Cards Are Punched

(1) Sort cards so that they are in order from largest to smallest according to one of the items (hereafter referred to as variable Y).

(2) With the cards in this order, next sort them so that they are in order from largest to smallest according to the second item (variable X). The cards are now in order with respect to both X and Y; that is, all cards with any given X digit are in order from large to small according to Y.

(3) Insert a blank card wherever any digit is missing in the X series in the range from the largest number in X through to zero. Then place the cards in the tabulator so that the card with the largest digit will go through first.

(4) Tabulate the following columns with a minor control on Y for a card count and intermediate control on X for sums:

X	Y	Card count	Sum of X	Sum of Y

(5) Tabulate the following progressive totals with an intermediate control on X, printing the following columns in this order:

X	Progressive card count	Progressive total of X	Progressive total of Y

(6) Remove the cards interpolated for missing X digits, sort a second time on variable Y, and insert blanks for any missing Y digits. Repeat step (5), but this time control on Y and print Y instead of X.

Subsequent Hand Computations

The following statistical data can then be computed. In step (4), N is the total card count, the tabulation being adjusted so as to omit blank cards; *mean* X is the final total *sum of* X divided by N; *mean* Y is the final total *sum of* Y divided by N; and a scattergram can be completed at once by copying from the several card counts to the corresponding spaces of the scattergram. In step (5) the sum of the *progressive totals of* X is the sum of squares for X, and the sum of the *progressive totals of* Y is the sum of the cross-

products, *XY*. In step (6) the sum of *progressive totals of Y* is the sum of squares for *Y*, and the sum of *progressive totals of X* is once again the sum of the cross-products, *XY*. Incidentally, the sum of the progressive card count will also be the sum of the variable which is the intermediate control. These data may then be combined by conventional formulas to yield the product-moment coefficient and the standard deviation for *X* and *Y*.

When zero is represented in the score range, the directions are modified to the extent that the sums of progressive totals do not include the progressive totals tabulated for the last, or zero, control card.

There is still some hand labor in translating the machine computations and tabulations to correlation results. Some installations may be able to handle part of that labor also. For example, if the tabulator is fitted with digit selectors, it will print the final scattergram directly (*2*). However, most offices are not equipped with expensive special features. The above operations require only a sorting machine and a numerical tabulator with a progressive-totals plug or switch and so can be carried out by any ordinary installation.

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Plastic Cages for Insects

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During the course of feeding and handling large numbers of mosquitoes used in the experimental transmission of malaria, it seemed desirable to find a more satisfactory cage. The ones formerly in use (*1*) were convenient but were relatively expensive and easily broken, being of hand-blown glass (Fig. 1).

Two companies (Lusteroid Container Company, South Orange, New Jersey, and Celluplastic Corporation, Newark, New Jersey) were found that made cages out of cellulose nitrate base material according to our size specifications.

The cages used are of two sizes: 2½ inches long by 1½ inches inside diameter, and 2 inches long by 1⅝ inches inside diameter. They are cylindrical in shape and open at both ends with the edges rolled back in a flange (Fig. 1). This flange serves as a barrier which holds a rubber band which in turn secures the bobbinet end coverings. The larger size holds up to five mosquitoes conveniently; the smaller size is for individual mosquitoes.

During the past two years about 9,000 of these cages made by the Lusteroid Container Company have been used with quite satisfactory results. The advantages of the plastic jar are that (a) there is practically no danger of breakage when it is dropped

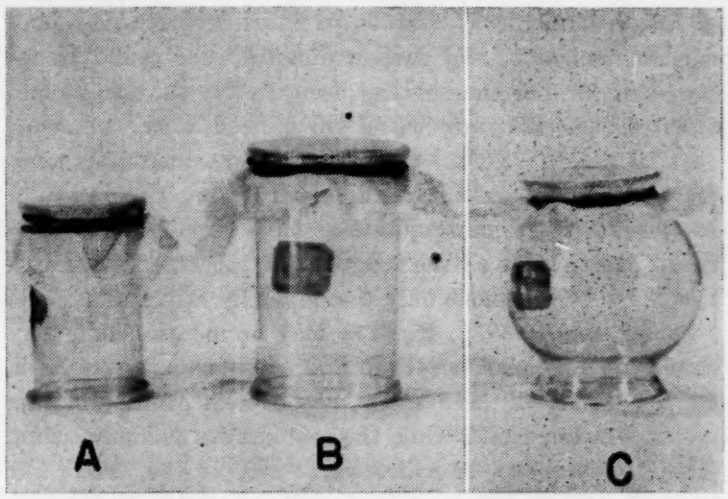

Fig. 1. Plastic and glass feeding jars with bobbinet in place on one end: A and B—small and large plastic jars; C—hand-blown glass jar.

or crushed—an important consideration when infectious insects are being handled or shipped; (b) the cost is low, less than $.04 each when purchased in large quantities, or about one-tenth the cost of the hand-blown glass cages; and (c) the jar is easily washed and stored.

The glass cages were heavier and stayed in position better during experimental feeding. However, with a little care the plastic jar was satisfactory. Also, the glass cages provided slight magnification of insects due to curvature of the sides. This was not significant, however, in the over-all appraisal.

The plastic cages are readily cleaned in either soapy cold water or alcohol. Some of the first cages received seemed to have a residuum toxic to mosquitoes, but this disappeared after thorough washing.

For over two years other types of plastic materials have been utilized in cage construction. Plastic screen, 16-mesh per inch, satisfactorily replaces galvanized screen for adult colony cages and does not rust.

Satisfactory emergence cages have been constructed of sheet plastic, 22 inches by 10 inches, bent into a semicylinder and tacked to a wooden base 10 inches square. The back of this cage is of plastic screen and the front is a muslin sleeve.

The above suggests the use of plastics instead of glass for various other types of insect cages.

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Letters to the Editor

On Tobacco Smoke

Mario Domingues de Campos has published a paper (*An. Fac. farm. odontol. Univ. São Paulo*, 1939–40, **1**, 15) on this subject which came to my attention only recently (*Chem. Abstr.*, 1945, **39**, 5395). He reports, apparently as his discovery, that pyrrole, pyridine, hydrocyanic acid, carbon monoxide, and carbon dioxide were found in tobacco smoke but that he has been unable to detect nicotine. These are statements which need some comment, for the presence of all these compounds, including nicotine, in tobacco smoke was detected a long time ago; in fact, quantitative determinations have already been made in all cases. Out of the long list of nicotine determinations in tobacco smoke only the paper by Barta and Toole (*Angew. Chem.*, 1932, **45**, 671) may be mentioned. They confirmed Lehmann's earlier findings (*Arch. Hig.*, 1909, **68**, 319) that, on the average, 93 per cent of the nicotine appears in the smoke while the rest suffers decomposition. Also, the quantity of hydrocyanic acid in the smoke has been determined repeatedly. Waser and Stähli (*Z. Unters. Lebensm.*, 1934, **67**, 280) confirmed Lehmann's earlier work, finding 0.020–0.0034 per cent of the tobacco weight as hydrocyanic acid in the main (interior) flow of smoke. It is worth noticing that the tobacco itself is free of hydrocyanic acid and that the quantity of the acid formed is independent of the nicotine content. Lehmann has also shown that tobacco smoke contains pyrrole. The writer (*Oesterr. Chem. Ztg.*, 1937, **40**, 434) has performed a series of quantitative determinations of pyrrole in tobacco smoke showing that the main flow contains 20–80 mg. per cent pyrrole. The quantity varies with the speed of smoking and increases with the humidity and the N-content of the tobacco but is independent of its nicotine content. This indicates clearly that pyrrole is not a decomposition product of the nicotine. It was concluded that it is formed by the thermal decomposition of the proteins of the tobacco, as Schützenberger and Bourgeois (*Bul. Soc. chim.*, 1876, 289) had observed this reaction in the case of the destructive distillation of isolated proteins. Also, the determination of pyridine bases in tobacco smoke has been dealt with in several papers; Preiss (*Pharm. Zentralhalle*, 1936, **29**, 437) has given a list of references.

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A Method for the Quantitative Estimation of DDT in Plant and/or Sulfur-containing Materials

The original dehydrohalogenation method (F. A. Gunther. *Ind. eng. Chem.* (Anal. ed.), 1945, **17**, 149–150) for DDT estimation may involve blanks considerably higher than the expected DDT residues and attributable to chloride-contaminated reagents and filter paper and to secondary effects of saponification, *i.e.* fatty acids responding to the silver titration. Sulfur also interferes, and since DDT-sulfur may become an important insecticide, this disadvantage may be serious.

If halogen-free reagents, removable by volatilization or by phase separation, could replace alcoholic potassium hydroxide, nitric acid, barium nitrate, etc., both the reagent and saponification blanks could be reduced. Reduction of reagent blank from 2 mg. to 0 mg. of DDT has been accomplished by substituting 4.5 N ammoniacal methanol as the dehydrohalogenating reagent, which eliminates the necessity for barium precipitation, reduces the amount of nitric acid for neutralization, and usually eliminates the problem of saponification products.

The procedure is adaptable to the determination of DDT in mixtures containing as much as 90 per cent sulfur.

Procedure. Weigh sufficient dry sample to contain 1 or more mg. of DDT, cover with measured amount of benzol, and set overnight. Pour off the extract through a double thickness of "Shark Skin" filter paper, measure the volume, transfer to an Erlenmeyer flask, and evaporate just to dryness as described by Gunther. Add 3 ml. of benzol to the residue with shaking, then 25 ml. of 4.5 N anhydrous ammoniacal methanol solution (for 125-ml. flask; use 50 ml. of reagent if flask is 500-ml. size), cap the flask with a collapsed finger stall, and hold for 16 hours in a 45° C. incubator.

Add 10 ml. of 3 per cent hydrogen peroxide solution and evaporate the ammonia and methanol, on a hot plate, with the aid of a jet of air. To residue add 40 ml. of distilled water and 1 ml. of 2 N nitric acid.

Remove sulfur not reacted with the ammonia solution by filtration (chloride-free paper). If noticeable oily material is present, transfer the sample to a separatory funnel and extract with 35 ml. of diethyl ether. Discard ether extract, and re-extract the aqueous layer with 35-ml. portions of petroleum ether until no more color is extracted.

Titrate chloride ion in the sample with 0.01 N silver nitrate and 0.01 N thiocyanate, according to Gunther, or by his procedure using the Leitz G & D Electro-titrator with 0.05 milliequivalent added sodium chloride present and deducted prior to the calculation which follows:

Each milliequivalent silver nitrate = 0.3545 grams of DDT.

Therefore:

(1) $[(\text{ml. } AgNO_3 \times N) - (\text{ml. } KSCN \times N)] \times 0.3545 =$ grams of DDT (gross)

(2) grams of DDT (gross) − blank (if present) = grams of DDT (net)

(3) $$\frac{\text{grams of DDT (net)} \times \text{total benzol volume} \times 10^6}{\text{volume filtered extract} \times \text{grams of sample}} =$$ DDT in ppm.

Known quantities from 0.002 to 0.184 gram of DDT, with or without sulfur present, carried through the entire procedure gave recoveries of 91–96 per cent, technical

rade containing some of the o,p′ isomer apparently reting the same as pure p,p′-DDT. In dried orange and lfalfa meals having zero blanks, added DDT up to ,000 ppm gave recoveries of the same order, 90–96 per ent. Routine use of the method on dried meal products rom experimentally sprayed crops has reproducibly indicated residues of 1–9 ppm.

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roperties of a Virus Inactivator From Yeast

A virus inactivator from yeast has been reported earlier y the undersigned (*Science*, 1942, **95**, 586–587). Simple ethods of isolating it and some of its properties have een described in the same publication. From the analyis of its constituent elements, the ratio of C, H, and O, nd some qualitative chemical tests, it was believed that he substance is a polysaccharide. Since these results ere reported, additional properties have been found nd are recorded here.

The virus inactivator was hydrolyzed by heating with per cent HCl or H_2SO_4 until foaming ceased (about 2 ours). The per cent reducing sugar calculated as glu-ose (Somogyi-Shaffer-Hartmann method) in the neutral-ed hydrolysate was 85 with HCl and 88 with H_2SO_4. sazones indistinguishable in appearance from glucos-zone were formed in abundance from the hydrolysate, urther supporting the view that the substance is com-osed largely of carbohydrates.

The 12–15 per cent noncarbohydrate residue suggested he possibility that the inactivator may be a glucoside. owever, the enzyme, β-glucosidase, prepared according o the procedure of Sumner and Howell (*Laboratory experiments in biological chemistry*. New York: Academic Press, 1944) from fresh almond meal, failed to hydrolyze it or to impair its activity against tobacco mosaic virus.

Longsworth scanning diagrams of a purified solution of inactivator run in a Tiselius electrophoresis cell at pH 7.5 showed but one boundary, indicating that the sample was electrophoretically homogeneous. A mixture of tobacco mosaic virus and a concentration of inactivator sufficient to render 98 per cent of the virus inactive showed two boundaries, one for excess inactivator and a second for inactive virus. A control scanning diagram of tobacco mosaic virus alone could be superimposed on the boundary of the inactive virus, showing that the net charge of the virus particle is not altered by the action of the inactivator. This fact is interpreted to indicate that a general adsorption phenomenon, in the sense that large areas of the virus particle are coated with the inactivator, is not involved; rather, the reaction is presumed to be more selective.

Electron micrographs (RCA Electron Microscope Model B) of purified tobacco mosaic virus which had been inactivated by the yeast inactivator showed no detectable evidence of disintegration or other gross change.

The above results provide further evidence that the inactivator is a polysaccharide and that inactivation is probably brought about by a reaction involving the inactivator and some group in the virus particle which is necessary for its infectivity.

A portion of this work was completed in the laboratories of the Departments of Plant Pathology and Biochemistry, New York State College of Agriculture, Cornell University, Ithaca, New York.

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Book Reviews

The new genetics in the Soviet Union. **P. S. Hudson and R. H. Richens. Cambridge, Engl.: Imperial Bureau of Plant Breeding and Genetics, 1946. Pp. 88. 6s.**

Here is a long-awaited and greatly needed study of the xtraordinary developments connected with the name of he Russian agronomist, T. D. Lysenko, from which arose he now famous Genetics Controversy which rocked Soviet iology and aroused the interest of the whole scientific orld. What was needed was a sober, careful description f the facts and a reasoned analysis of the interpretations hich gave rise to the controversy. This difficult task as been accomplished so well by the two British authors hat the importance of their book transcends the limits f this particular controversy and of genetics. It is a ontribution to the methodology of scientific discourse hich may be read with interest by scientists and phiosophers generally. Coming as it does on the heels of he appearance of Lysenko's chief theoretical treatise (*Heredity and its variability*. Translated by Th. Dobzhansky. New York: King's Crown Press, Columbia Univ., 1946; see *Science*, 1946, **103**, 180), it will hasten and facilitate the judgment of scientists on one of the most remarkable controversies of our time.

The study is based on an examination of the original publications, most of them in Russian, in which, between 1932 and 1944, appeared the experimental evidence, theoretical discussions, and polemics of the Lysenko school and its opponents. In addition, the sources of Lysenko's ideas have been traced by reference to the works of Darwin, Naudin, Timiriazev, Burbank, Michurin, and others. These citations, together with a few from modern non-Russian sources, bring the bibliography up to some 300 titles, each with complete listing of author, title, and source in original language and English. There is good evidence that these works were carefully combed and con-

scientiously used. All important statements attributed to the authors quoted are printed both in the original language (usually Russian) and in English, and much of the critical data are translated and reproduced in tabular form so that these can be judged by those unable to read or to consult the Russian original.

In all of this careful work the authors have maintained an objective, judicial, and respectful attitude against which the charge of prejudice cannot be sustained. For this reason the general verdict of "not proven," which they pronounce upon nearly all of the claims of the Lysenko school, will command respect both in the Soviet Union and in other countries.

It was a sound idea to begin this work of assessment by sketching some of the 19th-century views of the origin and transmission of hereditary variations, for after the recital of the speculations of Darwin and later horticulturists such as Michurin and Burbank, the reader finds nothing novel about Lysenko's ideas except the strange and unscientific language, the vehemence with which they are stated, and the vigor of his attack upon Mendelism. It even appears that the central corpus of Lysenkoism did not appear until he became associated with Prezent in 1935. Thereafter the authors generally refer to Lysenko and Prezent, attributing to the latter the chief dialectical and theoretical elaboration of Lysenko's system.

It was useful, too, to preface an examination of Lysenko's ideas by a discussion of the philosophy which animates them. Hudson and Richens' description of dialectical materialism and its application to natural science is a kind of tour de force of brevity and conciseness; and while many of the critical statements are inadequately justified, the authors have clearly stated the elements essential for understanding the dialectical basis of the work of Lysenko and Prezent and their school.

Western scientists may well be amazed by some of the methods of discourse adopted by that school, for there, in the midst of a society recently founded in revolution, the appeal to authority becomes a common device. "This erection of Darwin's work to the status of a canon and the grave distrust with which critics of its contents are regarded is one of the strangest developments of genetical science in Russia. It is impossible to avoid comparing this form of Darwinian exegesis with the extremely literal interpretation of the Bible practiced by Christian fundamentalists" (p. 25).

How Lysenko's followers used these methods is well illustrated in their attempt to discredit Mendelism, for they made of Mendelism, which has grown and changed a good deal since Mendel's time, a dogma in the image of their own rigid theory and then attacked it with the same weapons by which theirs is to be defended: appeal to authorities—Darwin, Michurin, Marx, Lenin; outlawing of heresy as antidialectical; impugning of motives of opponents and association of opponents' ideas with other repugnant views (Mendel-Morganists are bourgeois, fascist, believers in race inequality etc.); and appeal to practical usefulness. Lysenko's strength in Russia came from the fact that some exponents of Mendelism in other countries, e.g. Germany, were actually fascists, practiced race prejudice, and were not animated by a desire to have their science serve human needs. It is clear that his arguments often were not addressed to scientists in his own or other countries but to the mass of Russian peasants and workers who have responded by granting him great political power.

The main body of the book is concerned with a detailed presentation of the facts underlying Lysenko's theories under 12 headings ranging from genetics of earliness to graft hybridization, and with the interpretations applied. In every case except one it is concluded that the point is not proved or, if proved, is not new. The exception is graft hybridization, in which it is judged that "the evidence for genetic interaction between stock and scion is not compelling but suggestive. Further experiments are needed before a conclusion can be reached" (p. 51).

The interpretations of the Lysenko school are carefully examined. It is pointed out that "the whole of Lysenko's genetical system is permeated with the Darwinian notion that adaptation to environment is the key to the understanding of all biological variation. Conversely, as Lysenko and Prezent frequently point out, plants by becoming adapted to certain environmental conditions through natural selection, come, by means of this same process to have certain biological requirements in respect of the environment. Translating this concept into Lysenko's terminology, plants may be said to need or demand appropriate nutrients, this demand having arisen through natural selection. Extending this concept further, Lysenko states that this demand for certain nutrients may be further sharpened by natural selection so that, when various nutrients are present, the plant is able to absorb and assimilate those nutrients which are biologically advantageous and to reject the rest" (p. 58). This is the essence of Lysenko's nutrient theory, the central concept of plant development from which all his genetical ideas are derived. If it sounds more reasonable in these words of Hudson and Richens than in those in which Lysenko stated it in his 1943 paper, we have only to turn over a few pages to the section on analysis to find the British authors proving that not only does the theory lack factual support but has inconsistencies within itself and is actually antidialectical. Here the critics have met Lysenko and Prezent on their own ground and have borne away the palm.

This whole section may be read as one of the best examples yet provided of the interplay of Marxian dialectic upon scientific facts. Although the authors show that this effort has not produced a "New Genetics," they also show why such experiments in methodology which have infiltrated and conquered one section of Soviet agronomy are not to be summarily dismissed or disregarded. It is surely better for science that Lysenko's claims have now been exposed and rejected after a full and sober examination.

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